IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

Bernhard LINDENTHAL et al. Examiner: HUI, San-Ming R

Serial No.: 10/606,289 Group Art Unit: 1617

Filed: JUNE 26, 2003 Confirmation No.: 3274

Title: **METHOD FOR FERTILITY CONTROL**

DECLARATION UNDER 37 C.F.R. § 1.132

Sir:

I, Bernard LINDENTHAL, being duly warned, declare that:

My CV, which is attached herewith, demonstrates my expertise to sign this declaration.

I am a co-inventor of the subject matter disclosed in US application serial No. 10/606,289. As a result of German law, I will receive some compensation related to sales if the invention is ever commercialized.

The following experiments were conducted by me or under my supervision:

.....

Goal: Evaluation of a combination of COX-inhibitors and EP2-receptor antagonists.

Methods:

Within the ovary the LH surge prepares antral follicles for ovulation and fertilization of the oocytes. Cumulus cells surrounding the oocyte (COC, cumulus oocyte complexes) are a key factor for successful fertilization *in vivo*. Prior to ovulation and triggered by the LH surge, the tightly packed cumulus cells form an extracellular matrix leading to an expansion of the COCs, which is a prerequisite for ovulation and fertilization. The influence of compounds of the present application on this process was assessed by the following method:

In juvenile female mice (age: 18-22 days, C57 B1/6J x DBA/2J) F1, Charles River) follicular development was stimulated by a single injection (i.p.) of pregnant mare serum gonadotropin (PMSG, 10IU). Forty-eight hours after PMSG priming, mice were administered 10IU (i.p.) human

chorion gonadotropin (hCG, acting on the LH-receptor), which initiates ovulation after approximately 12h. Fourteen hours after hCG injection the animals were killed and COCs were recovered from the oviduct/bursa ovary. Collected COCs were used for *in vitro* fertilization (IVF).

To assess the *in vivo* effects of Cox-2 inhibitors and/or EP_2 antagonists on this process, the compounds were administered 8h before the hCG injection and together with hCG. IVF was performed without any test compounds in the media.

Assay details

In one experiment each group contained 5 animals. The COX-2 inhibitor (Rofecoxib) was given by p.o. application at doses of 1, 0.5 and 0.1 mg/animal (in Myristate/NaCl solution) 8h before hCG or together with the hCG injection. The EP2 antagonist (ZK6073610) was applied s.c. at a dose of 0.5 mg/animal (in benzylbenzoate/castor oil, 1:4 v/v). All animals received the same amounts of vehicles.

IVF was performed using 40000 sperms/0.5 ml for 1h. Thereafter COCs are washed free of sperms and cultivated over night. Fertilization is determined by the two cell stage. Fertilization rate is calculated in percent of used COCs. The experiment was replicated three times. For comparison of the three independent experiments the fertilization rates of the control groups are given the value of 1 (fertilization factor) and the fertilization factor of the treatment groups are calculated in relation to the fertilization factor of the control group (i.e., a fertilization factor of 0.5 in a treatment group means that 50% less COCs were fertilized compared to the control group).

Results:

The results are presented in Exhibit A, which is attached to this declaration.

The combined results of three independent experiments on the fertilization factor (given as mean ± SD) were analyzed. Whereas treatments with the Cox-2 inhibitor alone caused a significant dose-dependent decrease of the fertilization factor (*Student's t-test*, unpaired, two sided), the treatment with the EP2 antagonist alone did not show a significant effect. Combinations of the Cox-2 inhibitor and the EP2-antagonist also showed significant reduction of fertilization factor. Interestingly, a combination of the lowest tested dose of 0.1 mg of the Cox-2 inhibitor together with the EP2 antagonist showed a significantly lower fertilization factor not only compared to treatment with such low 0.1 mg dose of Cox-2 inhibitor, but even in comparison to the highest Cox-2 dose tested (1 mg).

These data show that an EP2-antagonist significantly enhances the effects of a Cox-2 inhibitor. Moreover, combinations of an EP2-antagonist with a Cox-2 inhibitor unexpectedly and significantly reduce the fertilization factor. In addition, the maximal *in vivo* effect achievable with a Cox-2 inhibitor can be increased by co-administration of an EP2-antagonist, even when low doses of Cox-2 inhibitors are administered.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

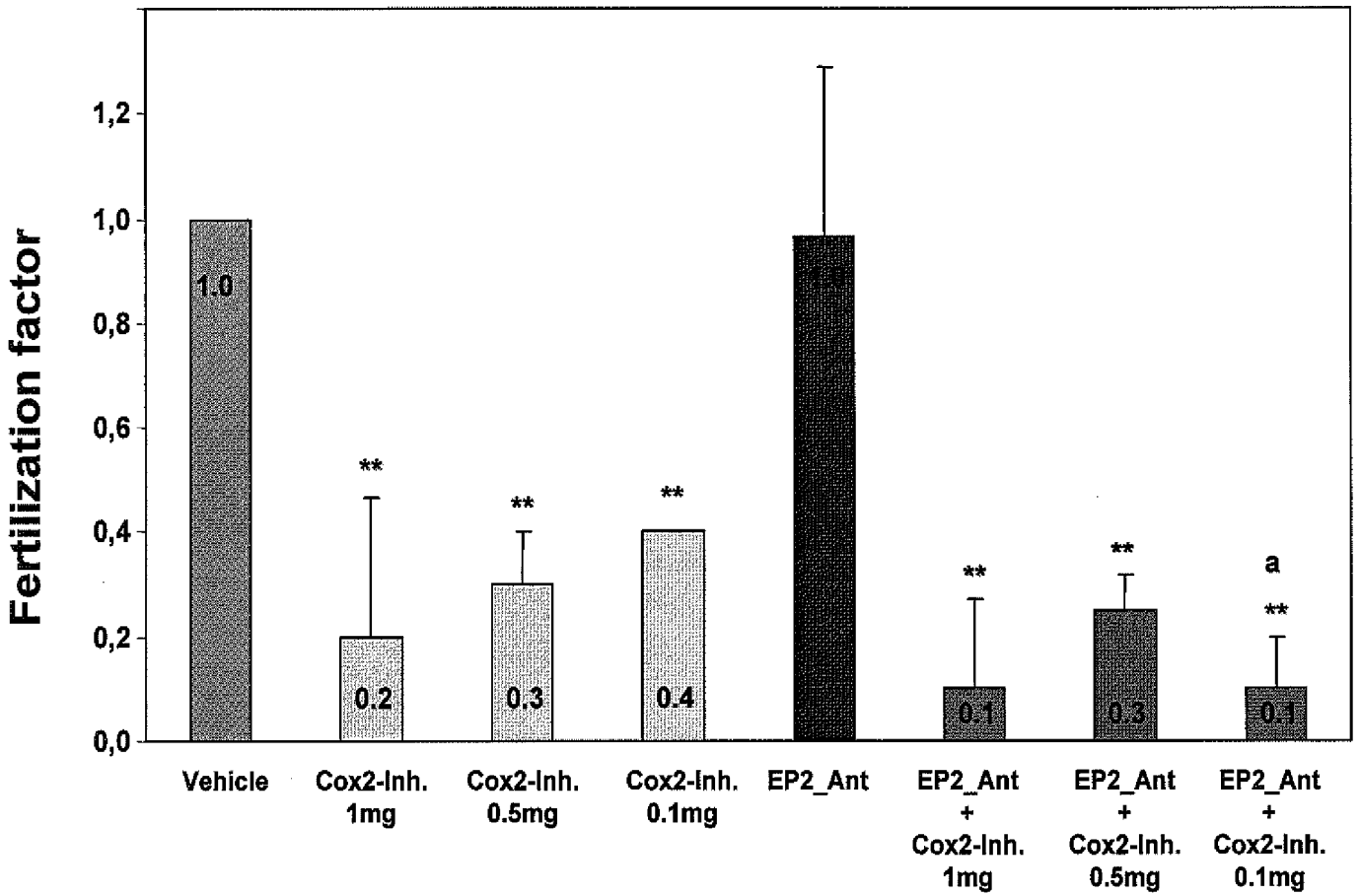
11. Sep. 2008

Date

Dr. Bernard Lindenthal

Combination of Cox-2 Inhibitor + EP2-Antagonist **Serial No.: 10/606,289**

- compounds were applied in vivo
- after ovulation: cumulus oocyte complexes were collected
-and incubated with sperm (without compounds)



EP2-Ant: 0.5mg/animal s.c., 2x

Cox-2-Inh: 1 – 0.1 mg/animal p.o., 2x

Values are means (± SD) from n=3 independent experiments

** $p<0.01$ vs vehicle

[a] $p<0,01$ vs Cox-2 Inh. 0.1mg

Combination of Cox-2 Inhibitor + EP2-Antagonist

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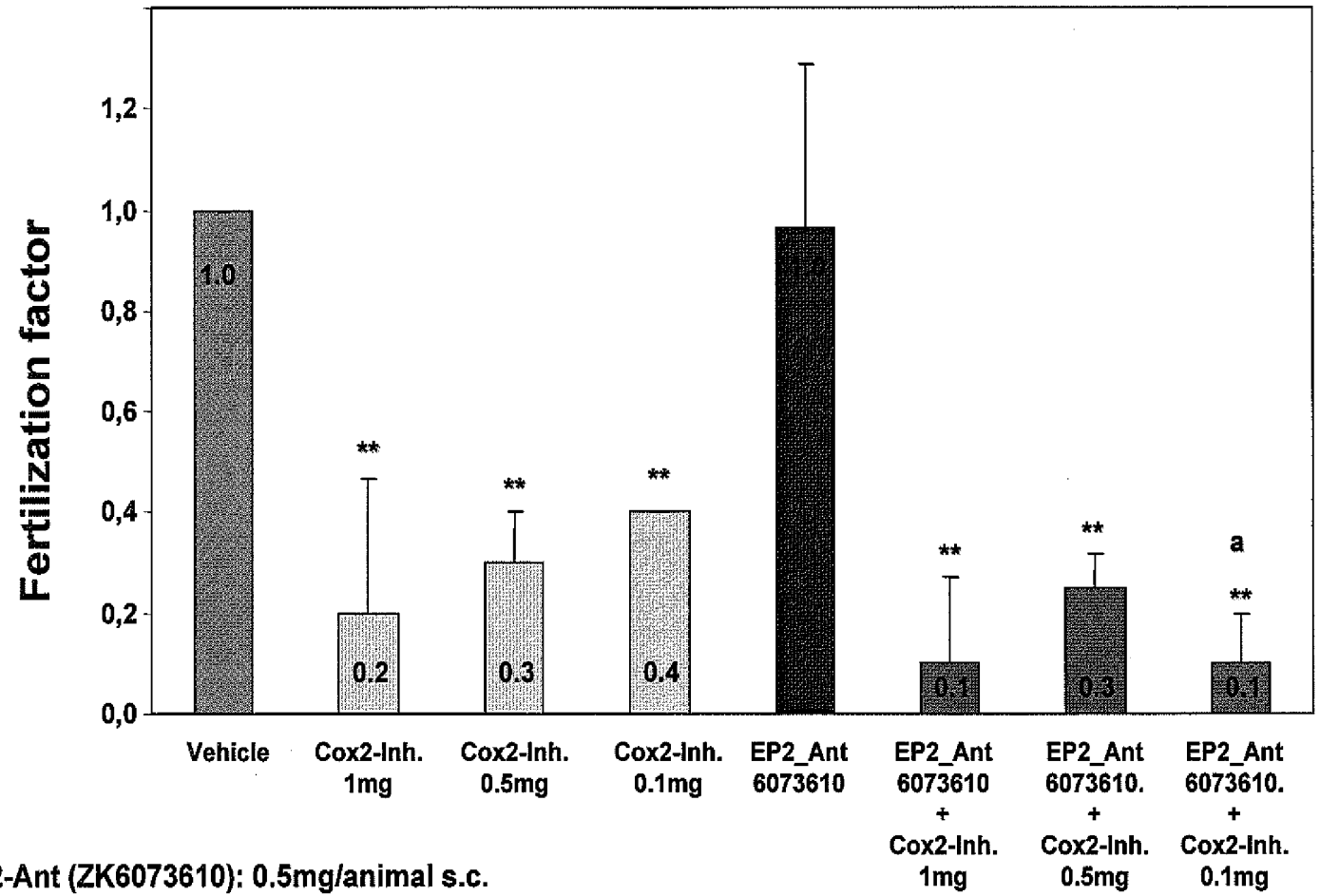
- after ovulation: cumulus oocyte complexes were collected and incubated with sperm (without compounds)



EP2-Ant (ZK6073610): 0.5mg/animal s.c.

Cox-2-Inh (ZK316075): 1 – 0.1 mg/animal p.o.

** p<0.01 vs vehicle

[a] p<0.01 vs Cox-2 Inh. 0.1mg

Values are means (± SD) from n=3 independent experiments